Mold allergen sensitization in adult asthma according to integrin β3 polymorphisms and Toll-like receptor 2/+596 genotype.

Lidwien A. M. Smit, Emmanuelle Bouzigon, Jean Bousquet, Nicole Le Moual, Rachel Nadif, Isabelle Pin, Mark Lathrop, Florence Demenais, Francine Kauffmann, Valérie Siroux

JACI 2011, in press

Mold allergen sensitization in adult asthma according to *ITGB3* polymorphisms and *TLR2*/+596 genotype

Lidwien A.M. Smit, PhD,[a-c] Emmanuelle Bouzigon, MD PhD,[d-f] Jean Bousquet, MD,[b,g] Nicole Le Moual, PhD,[b,c] Rachel Nadif, PhD,[b,c] Isabelle Pin, MD,[h-j] Mark Lathrop, PhD,[k] Florence Demenais, MD,[d-f] Francine Kauffmann, MD,[b,c] Valérie Siroux, PhD, [h,i] on behalf of the Epidemiological Study on the Genetics and Environment of Asthma

[a] Institute for Risk Assessment Sciences, Division Environmental Epidemiology, Utrecht University, The Netherlands;
[b] INSERM, CESP Centre for research in Epidemiology and Population Health, U1018, Respiratory and environmental epidemiology Team, F-94807, Villejuif, France;
[c] Université Paris Sud 11, UMRS 1018, F-94807,Villejuif, France;
[d] INSERM, U946, Paris, France;
[e] Université Paris Diderot, Paris 7, Institut Universitaire d'Hématologie, Paris, France;
[f] Fondation Jean Dausset-Centre d'Etude du Polymorphisme Humain (CEPH), Paris, France;
[g] Hôpital Arnaud de Villeneuve, Montpellier, France;
[h] INSERM, U823, Grenoble, France;
[i] Université Joseph Fourier- Grenoble 1, France;
[j] Centre Hospitalier Universitaire de Grenoble, Grenoble, France;
[k] Commissariat à l'Energie Atomique, Institut de Génomique, Centre National de Génotypage, Evry, France

Correspondence:
Lidwien Smit, Institute for Risk Assessment Sciences, Division Environmental Epidemiology
Utrecht Univeristy, P.O. Box 80178, Utrecht, The Netherlands
Tel: +31 302538696 Fax: +31 302539499
E-mail: L.A.Smit@uu.nl

Funding:
Supported by the French Ministry of Higher Education and Research, University Paris Diderot-Paris 7, grants from the French Agency for Environmental and Occupational Health Safety (grant AFSSET-APR-SE-2004), the French National Agency for Research (grants ANR 05-SEST-020-02/05-9-97 and ANR 06-CEBS), Merck Sharp & Dohme (MSD), Hospital program of clinical research (PHRC)-Paris, and by the Netherlands Organisation for Scientific Research (NWO) Van Gogh Programme for French-Dutch cooperation. Lidwien Smit was supported by a European Academy of Allergology and Clinical Immunology-Global Allergy and Asthma European Network (EAACI-GA^{2}LEN) exchange fellowship award.

Manuscript word count: 3130 words
Abstract word count: 249 words

ABSTRACT

Background: Integrin β3 (*ITGB3*) and Toll-like receptor 2 (*TLR2*) are candidate genes for asthma and sensitization to mold allergens. Integrin β3 forms a complex with TLR2, and this biological interaction is required for the response of monocytes to TLR2 agonists such as fungal glucan. **Objective**: To study whether genetic interaction between single nucleotide polymorphisms (SNPs) in genes encoding the TLR2-ITGB3 complex enhances susceptibility to mold sensitization. **Methods**: Association analysis was conducted in 1243 adults (524 with asthma) who participated in the follow-up of the Epidemiological Study on the Genetics and Environment of Asthma (EGEA). Allergic sensitization to mold allergens was determined by skin prick testing (SPT). Association of mold sensitization with 14 *ITGB3* SNPs was tested under an additive genetic model. Interaction between *ITGB3* SNPs and *TLR2*/+596, which was previously shown to be associated with asthma, was studied. **Results**: Positive SPT to mold was found in 115 subjects with asthma (22.0%), and in 61 subjects without asthma (8.5%). The *ITGB3* rs2056131 A allele was associated with mold sensitization in subjects with asthma with an odds ratio and 95% confidence interval of 0.60 (0.43-0.83), *P*=0.001. Ten other *ITGB3* SNPs were significantly associated with mold sensitization in *TLR2*/+596TT subjects with asthma (*P*=0.03 to 0.002), whereas much weaker associations were found in carriers of the *TLR2*/+596 C allele (*P*=0.60 to 0.04). Interaction between *TLR2*/+596 and these *ITGB3* SNPs was statistically significant (*P* interaction=0.05 to 0.001).
Conclusion: *TLR2/+596* genotype may influence the association between *ITGB3* SNPs and mold sensitization in adults with asthma.

Key Messages

- *TLR2/+596* genotype may influence the association between *ITGB3* SNPs and mold sensitization in adults with asthma.
- Findings in this population study are consistent with a biological interaction between integrin β3 and TLR2 in the innate immune response to fungal agents.

Capsule summary

This study sheds more light on the role of genetic variants in mold sensitization. Our results suggest that polymorphisms in *ITGB3* and *TLR2* interact to increase susceptibility to mold sensitization among subjects with asthma.

Key words

Alternaria; *Aspergillus*; allergy; asthma; *Cladosporium*; epidemiology; epistasis; genetics; innate immunity

Abbreviations

CI: Confidence interval
EGEA: Epidemiological study on the Genetics and Environment of Asthma
FDR: False discovery rate
GEE: Generalized estimating equations
ICS: Inhaled corticosteroids
ITGB3: Integrin β3
LD: Linkage disequilibrium
OR: Odds ratio
SNP: Single nucleotide polymorphism
SPT: Skin prick test
TLR2: Toll-like receptor 2

INTRODUCTION

Allergic sensitization to molds such as *Alternaria* and *Cladosporium* is a risk factor for asthma, asthma severity, and allergic rhinitis.[1-3] The prevalence of mold sensitization depends strongly on geographic and climatic conditions,[3, 4] and exposure to indoor mold allergens has been associated with mold allergy and asthma symptoms in children and adults.[5-8] Besides the influence of the environment, heritable factors were shown to contribute to mold sensitization as well. Concordance for skin prick testing to a mixture of *Alternaria* allergens was significantly greater among identical twins than for non-identical twins,[9] and maternal sensitization to *Alternaria alternata* significantly increased the risk of matched sensitization in their children.[10] Only a small number of candidate gene studies investigated the role of specific genetic variants in mold sensitization.[11-14] Weiss et al.[12] found that single nucleotide polymorphisms (SNPs) in the integrin β3 gene (*ITGB3*), which is located on chromosome 17q21.32, were associated with asthma and sensitization to mold allergens in Hutterites, a founder population, and in three outbred replication populations, whereas other allergens showed no association. A candidate gene study and a study that used genome-wide genotyping to assess the reproducibility of previously published asthma genes also found associations between *ITGB3* SNPs and asthma, but these studies did not investigate mold sensitization as an outcome.[15, 16]

ITGB3 encodes the beta chain of the receptor for a wide array of ligands including vitronectin and fibrinogen. Integrin β3 and its ligands play a key role in cell adhesion, cell proliferation and differentiation, platelet activation, and various other biological processes.[17] Vitronectin may participate in the remodelling process during lung development or response to injury by downregulating the expression of α-smooth muscle actin and reducing the contractile ability of human lung fibroblasts.[18]

Integrin β3 forms a complex with Toll-like receptor 2 (TLR2), and vitronectin and integrin β3 are required for the response of monocytes to bacterial lipopeptide and other TLR2 agonists such as fungal glucan.[19] We therefore hypothesized that variants in genes encoding the TLR2-ITGB3 complex may play a role in susceptibility to asthma and mold sensitization.

The present study is the first epidemiological study to address a gene-gene interaction between *ITGB3* and *TLR2*. In adults from the French Epidemiological study on the Genetics and Environment of Asthma (EGEA), the *TLR2*/+596 (rs3804099) C allele was associated with asthma in both case-control and family-based analyses.[20] We aimed to study whether *TLR2*/+596 genotype modified associations between *ITGB3* SNPs and asthma and sensitization to mold allergens. In addition, we aimed to confirm associations between *ITGB3* SNPs and asthma and mold sensitization.

METHODS

Population

The present analysis uses data from the 12-year follow-up of the EGEA survey. The design and protocol of EGEA, a family study and a case-control study of asthma, have been reported in detail elsewhere.[21, 22] Briefly, 2047 subjects were enrolled at baseline (1991-1995): 388 asthma patients (aged 7 to 70 years) from six chest clinics in five French cities, their 1244 first degree relatives, and 415 population-based controls. At follow-up (2003-2007), 92.2% of the alive cohort returned a self-completed questionnaire, and 77.1% completed a detailed questionnaire.[23] At the follow-up survey, all subjects were adults. For the present cross-sectional analysis, we used 1243 subjects (62.8% of the alive cohort) with complete data on asthma, mold sensitization, and genotyping (Figure E1 in the online supplement shows a flowchart). The 1243 subjects with complete data were slightly older, had more often studied at university level, and reported rhinitis more often than the 300 subjects who were excluded due to missing genotype or phenotype (mold sensitization) data (Supplementary Table E1). All participants gave written informed consent.

Health outcomes and exposure variables

Inclusion criteria used to define asthma in probands were based on self-reported answers to the four questions "Have you ever had attacks of breathlessness at rest with wheezing?", "Have you ever had asthma attacks?", "Was this diagnosis confirmed by a physician?", and "Have you had an asthma attack in the last 12 months?", or a positive response to at least two questions and a positive review of their medical record.[21] Asthma in relatives of probands was defined as a positive answer to at least one of the first two questions.[21]

Atopy was defined by the presence of a positive skin prick test (SPT) (mean wheal diameter ≥ 3 mm) to at least one of 11 aeroallergens (*Aspergillus*, *Cladosporium herbarum*, *Alternaria tenuis (=alternata)*, cat, *Dermatophagoides pteronyssinus*, *Blattella germanica*, olive, birch, *Parietaria judaica*, timothy grass, and ragweed pollen) using extracts made by Stallergènes (Antony, France). Mold sensitization was defined as a positive SPT to at least one of the 3 mold allergens. Mold species tested in EGEA were the same as in the study by Weiss et al.[12]

Environmental exposure to molds was assessed by questionnaire using items from the European Community Respiratory Health Survey.[6] A small lake near Montpellier was assumed to be a source of molds,[24] we therefore investigated recruitment in Montpellier as an environmental determinant of mold sensitization.

Genotyping

Fourteen SNPs in *ITGB3* (located at chromosome 17q21.32) with a minor allele frequency >5% were selected using a tagging approach. All SNPs were in Hardy-Weinberg equilibrium (P>0.01). Online Figure E2 shows linkage disequilibrium (LD) between SNPs. Genotyping was performed using Taqman Probes (Applied Biosystems, Foster City, CA) on an ABI7900HT Sequence Detection System at the Centre National de Génotypage (CNG, Evry, France).

Data analysis

Analyses using mold sensitization as an outcome were conducted in subjects with and without asthma separately, because mold sensitization is strongly associated with asthma, and subjects were recruited through patients with asthma. Determinants of mold sensitization were first explored by using univariate analyses (Chi-square test or t-test). All further analyses were done by generalized estimating equations (GEE model) to account for dependence among subjects sharing the same household. Odds ratios (ORs) and 95% confidence intervals (CIs) were adjusted for age and sex, unless stated otherwise. In the lack of evidence for a recessive or dominant genetic model, the effect of *ITGB3* SNPs on health outcomes was tested under an additive genetic model with the minor allele as risk allele.[12,15,16] To test whether association between *ITGB3* SNPs and health outcomes were modified by *TLR2/+596* (rs3804099) genotype (TT or CC+CT) we introduced a multiplicative gene-gene interaction term in the GEE model and used a generalized score test which follows a chi-square distribution with 1 degree of freedom. False Discovery Rate (FDR) adjusted P-values were calculated to take multiple comparisons (n=14 SNPs) into account.[25]

RESULTS

Association of mold sensitization and asthma

The present study comprised 524 subjects with asthma and 719 subjects without asthma. Mold sensitization was significantly more prevalent among subjects with asthma (n=115, 22.0%) than in subjects without asthma (n=61, 8.5%), with an adjusted OR (95%CI) of 2.80 (2.03-3.87). Exclusive mold sensitization was rare: only 13 (2.5%) subjects with asthma and 16 (2.2%) subjects without asthma were sensitized to mold without being sensitized to any of the other common allergens. *Alternaria, Cladosporium*, and *Aspergillus* sensitization were present in 71 (13.5%), 39 (7.4%), and 29 (5.5%) subjects with asthma and 27 (3.8%), 25 (3.5%), and 22 (3.1%) subjects without asthma, respectively. Atopy (sensitization to any of the 11 allergens tested) was found in 414

(79.0%) subjects with asthma, and in 277 (38.5%) subjects without asthma. Subjects with asthma who were sensitized to mold had more often used corticosteroids in the past year, and tended to have a lower age at the onset of asthma than those who were not sensitized to mold (52.6% vs. 40.1% and 13.1 y vs. 16.1 y, respectively; Table 1).

Determinants of mold sensitization

Table 1 shows determinants of mold sensitization in subjects with and without asthma (univariate analysis). Subjects with asthma who were sensitized to mold were younger, and more likely to be recruited in Montpellier than in the other centers. Among subjects without asthma, those sensitized to mold allergen were more often female, and recruited in Montpellier than those who were not sensitized to mold. Smoking habits and the presence of molds or water damage in the home were not associated with mold sensitization. Multiple regression models that included age, sex, and recruitment in Montpellier showed similar results as the univariate analyses. Recruitment in Montpellier was more strongly associated with sensitization to *Alternaria alternata* than to other mold allergens (OR 7.99 (3.24-19.66) and OR 4.18 (1.81-9.69), respectively).

Association of *ITGB3* SNPs, mold sensitization, asthma and atopy

The *ITGB3* rs2056131 A allele was associated with a lower prevalence of mold sensitization in subjects with asthma, with a frequency of 26% in mold sensitized subjects and 37% in subjects not sensitized to mold (OR 0.60 (0.43-0.83); Table 2). The P-value (0.001) was still statistically significant after correction for multiple testing. The same direction of association was shown for subjects without asthma, but the association was not significant (P=0.16). Among subjects with asthma, similar associations with *ITGB3* rs2056131 were found for each of the mold allergens: *Cladosporium* OR 0.49 (0.27-0.89), *Aspergillus* OR 0.47 (0.25-0.86), and *Alternaria* OR 0.70 (0.49-1.01). Excluding subjects from Montpellier, or adjusting for recruitment in Montpellier did not change results (OR 0.56 (0.39-0.81) and OR 0.57 (0.41-0.81), respectively). Correction for other potential confounders (educational level, use of corticosteroids, and smoking habits) did also not change results (OR 0.58 (0.37-0.90)). Analysis restricted to 414 atopic subjects also resulted in a similar OR (0.62 (0.45-0.86)). *ITGB3* SNPs were not associated with asthma or atopy (P>0.05; Supplementary Table E2 and E3).

Gene-gene interaction between *ITGB3* and *TLR2*

TLR2/+596 CC+CT was associated with asthma at the follow-up survey (OR 1.75 (1.36-2.26); P<0.0001), confirming the earlier reported association among adults at baseline.[20] *TLR2*/+596 CC+CT was not associated with mold sensitization in subjects with asthma (OR 1.13 (0.70-1.83) or subjects without asthma (OR 1.25 (0.70-2.24)). Among subjects with asthma, the associations between *ITGB3* SNPs and mold sensitization analyzed according to *TLR2*/+596 genotype showed statistically significant associations between 11 *ITGB3* SNPs and mold sensitization in *TLR2*/+596TT subjects (P=0.03 to 0.002), whereas much weaker or no associations were found in carriers of the *TLR2*/+596 C allele (P=0.60 to 0.04). Gene-gene interaction was statistically significant for 10 *ITGB3* SNPs (P interaction=0.05 to 0.001), and remained significant for 7 SNPs after adjusting for multiple comparisons (Table 3). Excluding subjects from Montpellier or adjusting for recruitment did not change these findings. The LD pattern (online Figure E2) shows that the SNPs involved in the interaction belong to different haplotype blocks. When a forward stepwise regression was applied in *TLR2*/+596TT subjects, two *ITGB3* SNPs that were not in LD (r^2=0.00) entered the model with P<0.05, suggesting that they were both independently associated with mold sensitization (rs15908 (V381V) and rs11079772 (3'UTR)). Both gene-gene interaction terms were statistically significant in a multiple regression model (rs15908 x *TLR2*/+596, p=0.02; rs11079772 x *TLR2*/+596, p=0.006; Figure 1). Among subjects without asthma, association between *ITGB3* SNPs and mold sensitization was not modified by *TLR2*/+596 (P interaction>0.05; Supplementary Table E4). Furthermore, *TLR2*/+596 did also not modify associations between *ITGB3* SNPs and asthma (P interaction>0.05; Supplementary Table E5) or atopy (P interaction>0.05; data not shown).

DISCUSSION

In the present study, we found significant associations between *ITGB3* SNPs and mold sensitization among adults with asthma, which were confined to carriers of the *TLR2*/+596TT genotype. Our findings suggest a gene-gene interaction between *ITGB3* and *TLR2*/+596 in mold allergen sensitization. These results are consistent with the biological interaction between integrin β3 and TLR2 that was recently demonstrated, suggesting an important role of the integrin β3-TLR2 complex in the innate immune response to fungal agents.[19]

Testing associations of multiple *ITGB3* SNPs and three phenotypes may have resulted in chance findings, which is a limitation of our study. However, our finding of epistasis between *ITGB3* and *TLR2* was still significant for seven *ITGB3* SNPs after correction for multiple testing. Another limitation is that skin tests were performed

with mold extracts which were not fully standardized and some subjects with mold allergy may have been missed. The allergen extracts were however of similar quality as those tested by Weiss et al.[12] The biochemistry of mold allergens is still poorly understood, and the concordance between skin prick test (SPT) and specific serum IgE results for individual molds is much lower than for non-fungal allergens.[26]

Interestingly, *TLR2*/+596 T/C did not modify associations between *ITGB3* SNPs and asthma or atopy, suggesting that the gene-gene interaction between *TLR2* and *ITGB3* may be specific for the response to molds. Weiss et al. also found associations between *ITGB3* SNPs and allergic sensitization to molds, but not to other common allergens.[12] It is not clear whether our results concern one or more specific mold species, or rather mold allergens in general. We found similar risk estimates for the association between *ITGB3* rs2056131 and each of the three molds studied, but there was insufficient statistical power to study the interaction between *TLR2* and *ITGB3* for allergic sensitization to each of the three individual mold species.

Multiple regression analysis showed that at least two *ITGB3* SNPs, rs15908 (V381V) and rs11079772, were independently involved in the interaction with *TLR2*. Rs2015729, a noncoding *ITGB3* SNP in high LD with rs15908, was previously associated with decreased *ITGB3* expression levels, which may provide a potential mechanism by which noncoding *ITGB3* variants have a functional effect.[27] Another functional *ITGB3* SNP (rs5918, Leu33Pro) has been associated with various effects, including enhanced thrombin formation,[28] coronary heart disease,[29] and cancer susceptibility.[30] *ITGB3* Leu33Pro was not genotyped in the present study, but it was in LD (r^2=0.76) with rs10514919, a SNP that showed a significant interaction with *TLR2* (P=0.05). Besides main effects of *ITGB3* variants on various clinical outcomes, three studies have presented evidence for epistasis between *ITGB3* and the serotonin transporter gene *SLC6A4* in autism susceptibility [27, 31, 32] and in serotonin levels.[31] Altogether, these findings suggest that multiple functional *ITGB3* SNPs may have different pleiotropic effects, possibly in interaction with other genes.

We can only speculate on the mechanism by which the effect of *ITGB3* SNPs on mold sensitization is modified by *TLR2*/+596. A recent study has shown that peripheral blood leukocytes of *TLR2*/+596 TT subjects produce significantly less cytokines in response to bacterial lipoprotein stimulation than those of *TLR2*/+596 CT and CC subjects.[33] Moreover, *TLR2*/+596 TT was the only *TLR2* variant associated with a lower sepsis morbidity rate.[33] The *TLR2*/+596 C allele was associated with asthma in both case-control and family-based analyses among EGEA adults, which is consistent with a functional role of *TLR2*/+596.[20] However, the exact functional significance of *TLR2*/+596 is so far unknown. A possible explanation could be a different expression of TLR2 on the surface of innate immune cells among subjects carrying the TT genotype compared with subjects carrying the C allele. In subjects with certain functional *ITGB3* SNPs, a modified TLR2 expression may potentially result in a decreased function of the ITGB3-TLR2 complex. Finally, it can be hypothesized that this altered function could lead to a less effective response to fungal agents resulting in an increased risk of mold sensitization.

We did not replicate associations between *ITGB3* variants and asthma.[12, 15] In a recent study that used genome-wide genotyping to assess reproducibility of SNPs in 39 previously reported asthma genes, only 10 SNPs in 6 genes were significantly associated with childhood asthma, including one SNP in *ITGB3*, which was not included in the present study.[15] Our study had sufficient statistical power (80%) to detect a relative risk (RR) between 1.32 and 1.40 for the association between *ITGB3* SNPs and asthma, and a RR between 1.66 and 1.82 for the association between *ITGB3* SNPs and mold sensitization in asthmatics, depending on disease allele frequency (varying between 0.19 and 0.40), and using the conservative Bonferroni correction for multiple comparisons. These RR seem reasonable and suggest that the lack of association cannot be attributed to a lack of power in our study. Replicating asthma susceptibility genes is a major challenge, and failure to replicate may be caused by heterogeneity between studies. Replicating interactions appears even more complicated.[34,35] Findings of our epidemiological study were analogous to a previously reported biological interaction between integrin β3 and TLR2.[19] In addition, we have attempted to replicate our findings in the population of adult Hutterites.[12] However, in that population only 93 subjects carried *TLR2*/+596 TT, and only 42 subjects were sensitized to mold (Dr. C. Ober, personal communication). Thus, there was no sufficient power to study interactions in this population. Moreover, Hutterites in general have a mild form of asthma, with corticosteroid use being quite rare. Other populations that were considered had not assessed sensitization to (the same) mold allergens. Variation between studies in asthma definition, age at onset, ethnicity, and other characteristics of the study population may reduce reproducibility. Furthermore, the influence of environmental exposures may also obscure genetic associations. For example, consistent associations between *CD14*/-260 and asthma or allergic sensitization were observed only after taking environmental endotoxin levels into account.[36] The present study would have benefited from measured fungal allergen and/or glucan levels in settled house dust. Due to the lack of objectively assessed environmental exposure, we cannot exclude the possibility that gene-environment interactions play an additional role in association of *ITGB3* and asthma or mold sensitization. In the present study we found an expected higher prevalence of mold sensitization in subjects recruited in Montpellier, where environmental exposure to mold was elevated.[24] However, excluding subjects from Montpellier, or adjusting for recruitment did not affect the results. In US homes, *Alternaria* antigen concentrations were higher in homes where either residents or field workers observed signs of mold or dampness,[37] and exposure to *Alternaria* was

associated with asthma symptoms.[8] We also investigated water damage and visible mold in the home as self-reported mold exposures, but these were not associated with mold sensitization.
We did not study severity of asthma as another phenotype, although mold sensitization is known to be associated with a more severe asthma phenotype.[1] In adult asthma patients from EGEA who did not use inhaled corticosteroids (ICS), mold sensitization was significantly associated with an increased risk for uncontrolled asthma (OR, 95% CI of 3.15, 1.02-9.78), but not for partly controlled asthma.[23] However, mold sensitization was not associated with an increased risk for uncontrolled asthma in ICS users.[23] The number of subjects did not allow further stratification for ICS use, or stratification for multiple levels of asthma control or asthma severity, and therefore we decided not to study associations between uncontrolled asthma or asthma severity and *ITGB3* genotype in the present study.
In conclusion, our epidemiological study suggests that genetic variants in *ITGB3* and *TLR2* interact to increase susceptibility to mold sensitization among subjects with asthma. Following an experimental study that demonstrated the essential role of integrin β3 in TLR2 signaling,[19] our population study was the first to show this specific gene-gene interaction, which may influence the demonstrated biological interactions. Future studies need to replicate these findings, and functional experiments are needed to assess the mechanism by which *ITGB3* and *TLR2* SNPs influence susceptibility to mold sensitization.

Acknowledgements
We thank Dr. Carole Ober and Dr. Emma Thompson for their helpful comments.

EGEA cooperative group
Coordination: F Kauffmann; F Demenais (genetics); I Pin (clinical aspects). ***Respiratory epidemiology***: Inserm U 700, Paris M Korobaeff (Egea1), F Neukirch (Egea1); Inserm U 707, Paris: I Annesi-Maesano; Inserm CESP/U 1018, Villejuif: F Kauffmann, N Le Moual, R Nadif, MP Oryszczyn; Inserm U 823, Grenoble: V Siroux ***Genetics***: Inserm U 393, Paris: J Feingold; Inserm U 946, Paris: E Bouzigon, F Demenais, MH Dizier; CNG, Evry: I Gut , M Lathrop. ***Clinical centers***: Grenoble: I Pin, C Pison; Lyon: D Ecochard (Egea1), F Gormand, Y Pacheco; Marseille: D Charpin (Egea1), D Vervloet; Montpellier: J Bousquet; Paris Cochin: A Lockhart (Egea1), R Matran (now in Lille); Paris Necker: E Paty, P Scheinmann; Paris-Trousseau: A Grimfeld, J Just. ***Data and quality management***: Inserm ex-U155 (Egea1), Paris: J Hochez; Inserm CESP/U 1018, Villejuif: N Le Moual, Inserm ex-U780, Villejuif: C Ravault; Inserm ex-U794, Evry: N Chateigner; Grenoble: J Ferran.

REFERENCES

1. Zureik M, Neukirch C, Leynaert B, Liard R, Bousquet J, Neukirch F*, et al*. Sensitisation to airborne moulds and severity of asthma: cross sectional study from European Community respiratory health survey. BMJ 2002;325:411-4.
2. Randriamanantany ZA, Annesi-Maesano I, Moreau D, Raherison C, Charpin D, Kopferschmitt C*, et al*. Alternaria sensitization and allergic rhinitis with or without asthma in the French Six Cities Study. Allergy 2010;65:368-75.
3. Halonen M, Stern DA, Wright AL, Taussig LM, Martinez FD. Alternaria as a major allergen for asthma in children raised in a desert environment. Am J Respir Crit Care Med 1997;155:1356-61.
4. Bousquet PJ, Chinn S, Janson C, Kogevinas M, Burney P, Jarvis D*, et al*. Geographical variation in the prevalence of positive skin tests to environmental aeroallergens in the European Community Respiratory Health Survey I. Allergy 2007;62:301-9.
5. Brunekreef B, Dockery DW, Speizer FE, Ware JH, Spengler JD, Ferris BG. Home dampness and respiratory morbidity in children. Am Rev Respir Dis 1989;140:1363-7.
6. Zock JP, Jarvis D, Luczynska C, Sunyer J, Burney P, European Community Respiratory Health Survey. Housing characteristics, reported mold exposure, and asthma in the European Community Respiratory Health Survey. J Allergy Clin Immunol 2002;110:285-92.
7. Verhoeff AP, van Strien RT, van Wijnen JH, Brunekreef B. Damp housing and childhood respiratory symptoms: the role of sensitization to dust mites and molds. Am J Epidemiol 1995;141:103-10.
8. Salo PM, Arbes SJ Jr, Sever M, Jaramillo R, Cohn RD, London SJ, *, et al*. Exposure to Alternaria alternata in US homes is associated with asthma symptoms. J Allergy Clin Immunol 2006;118:892-8.

9. Karihaloo C, Tovey ER, Mitakakis TZ, Duffy DL, Britton WJ. Evidence for the genetic control of immunoglobulin E reactivity to the allergens of Alternaria alternata. Clin Exp Allergy 2002;32:1316-22.
10. Misiak RT, Wegienka G, Havstad S, Ownby DR, Johnson CC, Zoratti EM. Specific allergic sensitization in parents and their 18-year-old offspring in the Suburban Detroit Childhood Allergy Study. J Allergy Clin Immunol 2009;123:1401-6.
11. Leung TF, Tang NL, Lam CW, Li AM, Fung SL, Chan IH*, et al*. RANTES G-401A polymorphism is associated with allergen sensitization and FEV1 in Chinese children. Respir Med 2005;99:216-9.
12. Weiss LA, Lester LA, Gern JE, Wolf RL, Parry R, Lemanske RF*, et al*. Variation in ITGB3 is associated with asthma and sensitization to mold allergen in four populations. Am J Respir Crit Care Med 2005;172:67-73.
13. Donfack J, Schneider DH, Tan Z, Kurz T, Dubchak I, Frazer KA*, et al*. Variation in conserved non-coding sequences on chromosome 5q and susceptibility to asthma and atopy. Respir Res 2005;6:145.
14. Carvalho A, Pasqualotto AC, Pitzurra L, Romani L, Denning DW, Rodrigues F. Polymorphisms in toll-like receptor genes and susceptibility to pulmonary aspergillosis. J Infect Dis 2008;197:618-21.
15. Rogers AJ, Raby BA, Lasky-Su JA, Murphy A, Lazarus R, Klanderman BJ*, et al*. Assessing the reproducibility of asthma candidate gene associations, using genome-wide data. Am J Respir Crit Care Med 2009;179:1084-90.
16. Thompson EE, Pan L, Ostrovnaya I, Weiss LA, Gern JE, F. LR,Jr*, et al*. Integrin beta 3 genotype influences asthma and allergy phenotypes in the first 6 years of life. J Allergy Clin Immunol 2007;119:1423-9.
17. Hynes RO. Integrins: bidirectional, allosteric signaling machines. Cell 2002;110:673-87.
18. Scaffidi AK, Moodley YP, Weichselbaum M, Thompson PJ, Knight DA. Regulation of human lung fibroblast phenotype and function by vitronectin and vitronectin integrins. J Cell Sci 2001;114:3507-16.
19. Gerold G, Ajaj KA, Bienert M, Laws HJ, Zychlinsky A, de Diego JL. A Toll-like receptor 2-integrin beta3 complex senses bacterial lipopeptides via vitronectin. Nat Immunol 2008;9:761-8.
20. Smit LA, Siroux V, Bouzigon E, Oryszczyn MP, Lathrop M, Demenais F*, et al*. CD14 and toll-like receptor gene polymorphisms, country living, and asthma in adults. Am J Respir Crit Care Med 2009;179:363-8.
21. Kauffmann F, Dizier MH, Pin I, Paty E, Gormand F, Vervloet D*, et al*. Epidemiological study of the genetics and environment of asthma, bronchial hyperresponsiveness, and atopy: phenotype issues. Am J Respir Crit Care Med 1997;156:S123-9.
22. Kauffmann F, Dizier MH. EGEA (Epidemiological study on the Genetics and Environment of Asthma, bronchial hyperresponsiveness and atopy)--design issues. EGEA Co-operative group. Clin Exp Allergy 1995;25:19-22.
23. Siroux V, Boudier A, Bousquet J, Bresson JL, Cracowski JL, Ferran J*, et al*. Phenotypic determinants of uncontrolled asthma. J Allergy Clin Immunol 2009;124:681-7.
24. Kauffmann F, Oryszczyn MP, Maccario J. The protective role of country living on skin prick tests, immunoglobulin E and asthma in adults from the epidemiological study on the genetics and environment of asthma, bronchial hyper-responsiveness and atopy. Clin Exp Allergy 2002;32:379-86.
25. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Statist Soc B 1995;57:289-300.
26. O'Driscoll BR, Powell G, Chew F, Niven RM, Miles JF, Vyas A*, et al*. Comparison of skin prick tests with specific serum immunoglobulin E in the diagnosis of fungal sensitization in patients with severe asthma. Clin Exp Allergy 2009;39:1677-83.
27. Weiss LA, Ober C, Cook EH,Jr. ITGB3 shows genetic and expression interaction with SLC6A4. Hum Genet 2006;120:93-100.
28. Undas A, Brummel K, Musial J, Mann KG, Szczeklik A. Pl(A2) polymorphism of beta(3) integrins is associated with enhanced thrombin generation and impaired antithrombotic action of aspirin at the site of microvascular injury. Circulation 2001;104:2666-72.
29. Rossouw JE, Cushman M, Greenland P, Lloyd-Jones DM, Bray P, Kooperberg C*, et al*. Inflammatory, lipid, thrombotic, and genetic markers of coronary heart disease risk in the women's health initiative trials of hormone therapy. Arch Intern Med 2008;168:2245-53.
30. Bojesen SE, Tybjaerg-Hansen A, Nordestgaard BG. Integrin beta3 Leu33Pro homozygosity and risk of cancer. J Natl Cancer Inst 2003;95:1150-7.
31. Coutinho AM, Sousa I, Martins M, Correia C, Morgadinho T, Bento C*, et al*. Evidence for epistasis between SLC6A4 and ITGB3 in autism etiology and in the determination of platelet serotonin levels. Hum Genet 2007;121:243-56.

32. Ma DQ, Rabionet R, Konidari I, Jaworski J, Cukier HN, Wright HH, *et al*. Association and gene-gene interaction of SLC6A4 and ITGB3 in autism. Am J Med Genet B Neuropsychiatr Genet 2010;153B:477-83.
33. Chen KH, Gu W, Zeng L, Jiang DP, Zhang LY, Zhou J, *et al*. Identification of haplotype tag SNPs within the entire TLR2 gene and their clinical relevance in patients with major trauma. Shock 2011;35:35-41.
34. Ober C, Vercelli D. Gene-environment interactions in human disease: nuisance or opportunity? Trends Genet 2011; in press.
35. Ege MJ, Strachan DP, Cookson WO, Moffatt MF, Gut I, Lathrop M, Kabesch M, Genuneit J, Büchele G, Sozanska B, Boznanski A, Cullinan P, Horak E, Bieli C, Braun-Fahrländer C, Heederik D, von Mutius E; GABRIELA Study Group. Gene-environment interaction for childhood asthma and exposure to farming in Central Europe. J Allergy Clin Immunol. 2011;127:138-44, 144.e1-4.
36. Simpson A, Martinez FD. The role of lipopolysaccharide in the development of atopy in humans. Clin Exp Allergy 2010;40:209-23.
37. Salo PM, Yin M, Arbes SJ Jr, Cohn RD, Sever M, Muilenberg M, *et al*. Dustborne Alternaria alternata antigens in US homes: results from the National Survey of Lead and Allergens in Housing. J Allergy Clin Immunol 2005;116:623-9.

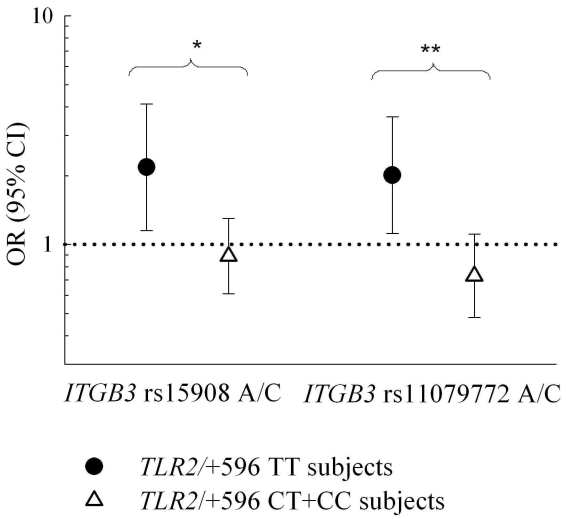

Figure 1. Association of two *ITGB3* SNPs (rs15908 A/C and rs11079772 A/C) with mold sensitization in a multiple regression analysis, stratified by *TLR2*/+596 genotype. *ITGB3* SNPs were tested under an additive model among subjects with asthma. *P value for interaction <0.05; **P value for interaction <0.01.

Table 1. Asthma characteristics and determinants of mold sensitization in 524 adults with asthma and 719 adults without asthma.

	Subjects with asthma				Subjects without asthma			
	Data available for n subjects	Mold SPT-	Mold SPT+	*P*	Data available for n subjects	Mold SPT-	Mold SPT+	*P*
N	524	409	115		719	658	61	
Sex, n (%) men	524	212 (51.8)	65 (56.5)	0.37	719	312 (47.4)	20 (32.8)	0.03
Age (y), mean ± sd	524	39.9 ± 16.8	36.3 ± 16.5	0.04	719	46.5 ± 16.3	43.4 ± 16.2	0.16
Status at inclusion	524			0.06	719			0.57
Probands, n (%)		188 (46.0)	68 (59.1)			0 (0)	0 (0)	
Relatives, n (%)		182 (44.5)	39 (33.9)			383 (58.2)	39 (63.9)	
Spouses, n (%)		4 (1.0)	2 (1.7)			67 (10.2)	4 (6.6)	
Controls, n (%)		35 (8.6)	6 (5.2)			208 (31.6)	18 (29.5)	
Recruited in Montpellier, n (%)	524	19 (4.7)	18 (15.7)	<0.0001	719	33 (5.0)	10 (16.4)	0.0003
Smoking habits	519			0.70	711			0.45
Never, n (%)		204 (50.4)	58 (50.9)			339 (52.1)	32 (52.5)	
Former, n (%)		106 (26.2)	26 (22.8)			166 (25.5)	19 (31.2)	
Current, n (%)		95 (23.5)	30 (26.3)			146 (22.4)	10 (16.4)	
Education at university level, n (%)	518	197 (48.8)	63 (55.3)	0.22	710	304 (46.7)	39 (66.1)	0.004
Living in a rural commune before 16y, n (%)	511	156 (39.0)	39 (35.1)	0.46	696	238 (37.3)	15 (26.3)	0.10
Water damage last year, n (%)	429	38 (11.3)	12 (12.9)	0.67	590	59 (11.0)	9 (17.7)	0.15
Mold in any surface inside the home last year, n (%)	457	72 (20.1)	17 (17.4)	0.55	619	100 (17.6)	9 (17.3)	0.95
Asthma onset (y), mean ± sd	492	16.1 ± 15.7	13.1 ± 14.2	0.07	--	--	--	--
FEV1 % predicted, mean ± sd	523	92.5 ± 17.4	91.5 ± 18.8	0.60	--	--	--	--
Inhaled corticosteroids in the past 12 months, n (%)	520	163 (40.1)	60 (52.6)	0.02	--	--	--	--
Active asthma*, n (%)	522	275 (67.6)	87 (75.6)	0.10				

*Active asthma: asthma attack or asthma treatment in the past 12 months

-- Data only shown for subjects with asthma

Table 2. Association of *ITGB3* SNPs with mold sensitization under an additive model in 524 adults with asthma and 719 adults without asthma.

					Subjects with asthma	Subjects without asthma
ITGB3 SNP	Position	Region	Alleles[†]	MAF	OR (95% CI)	OR (95% CI)
rs2317385	42684681	5'UTR	G/A	0.19	1.21 (0.83-1.77)	1.14 (0.74-1.76)
rs2056131	42688742	intron 1	G/A	0.33	0.60 (0.43-0.83)*	0.75 (0.51-1.12)
rs4525555	42692948	intron 1	C/T	0.33	1.09 (0.80-1.48)	1.05 (0.72-1.53)
rs3892084	42695420	intron 1	G/A	0.19	1.04 (0.72-1.49)	0.91 (0.57-1.46)
rs10514919	42697128	intron 1	G/T	0.26	1.07 (0.76-1.51)	1.12 (0.73-1.72)
rs8074094	42703020	intron 1	T/C	0.28	1.10 (0.79-1.53)	1.16 (0.77-1.73)
rs3851806	42705918	intron 1	G/C	0.18	1.05 (0.72-1.53)	0.92 (0.56-1.50)
rs2015729	42709492	intron 2	G/A	0.40	1.17 (0.87-1.58)	1.04 (0.73-1.48)
rs2292699	42717294	intron 4	C/T	0.38	1.13 (0.83-1.53)	1.02 (0.71-1.47)
rs15908	42723336	exon 9 V381V	A/C	0.38	1.12 (0.83-1.52)	1.03 (0.71-1.50)
rs2292863	42724129	intron 9	C/G	0.30	1.07 (0.77-1.49)	1.10 (0.74-1.62)
rs3809863	42740011	intron 14	C/T	0.47	1.03 (0.77-1.39)	1.16 (0.85-1.59)
rs11650072	42748664	3'UTR	C/T	0.33	0.91 (0.66-1.25)	0.95 (0.67-1.35)
rs11079772	42748738	3'UTR	A/C	0.29	0.93 (0.67-1.29)	1.01 (0.69-1.47)

*FDR-corrected P<0.05; [†]Major/Minor allele; MAF, minor allele frequency.

Table 3. Association of *ITGB3* SNPs with mold sensitization under an additive model in adults with asthma, according to *TLR2*/+596 genotype.

		Carriers of TLR2/+596 TT (n=126)			Carriers of TLR2/+596 CT or CC (n=362)			
ITGB3 SNP	Alleles[†]	Minor allele in mold SPT- subjects, n (%)	Minor allele in mold SPT+ subjects, n (%)	OR (95% CI)	Minor allele in mold SPT- subjects, n (%)	Minor allele in mold SPT+ subjects, n (%)	OR (95% CI)	P interaction
rs2317385	G/A	37 (19)	11 (21)	1.19 (0.58-2.43)	101 (18)	34 (21)	1.24 (0.79-1.97)	0.91
rs2056131	G/A	82 (41)	10 (19)	0.36 (0.18-0.72)	202 (36)	46 (29)	0.70 (0.47-1.03)	0.09
rs4525555	C/T	54 (27)	23 (44)	1.94 (1.04-3.63)	185 (33)	47 (30)	0.89 (0.61-1.30)	0.04
rs3892084	G/A	32 (16)	10 (19)	1.17 (0.54-2.55)	111 (20)	31 (19)	1.01 (0.65-1.55)	0.75
rs10514919	G/T	44 (22)	19 (37)	1.89 (1.05-3.40)	145 (26)	36 (23)	0.83 (0.52-1.33)	0.05
rs8074094	T/C	47 (24)	21 (40)	2.08 (1.16-3.73)	158 (28)	40 (25)	0.85 (0.55-1.32)	0.02*
rs3851806	G/C	33 (17)	11 (21)	1.29 (0.63-2.62)	105 (19)	30 (19)	0.99 (0.63-1.56)	0.55
rs2015729	G/A	66 (33)	30 (58)	2.79 (1.46-5.33)	231 (41)	61 (38)	0.91 (0.63-1.31)	0.003*
rs2292699	C/T	62 (31)	28 (54)	2.43 (1.28-4.62)	218 (39)	57 (36)	0.90 (0.62-1.31)	0.009*
rs15908	A/C	62 (31)	26 (54)	2.42 (1.24-4.70)	219 (39)	57 (36)	0.89 (0.61-1.29)	0.01*
rs2292863	C/G	53 (27)	22 (42)	1.85 (1.03-3.33)	175 (31)	44 (28)	0.83 (0.54-1.27)	0.04
rs3809863	C/T	103 (52)	15 (29)	0.37 (0.18-0.77)	252 (45)	86 (54)	1.38 (0.96-1.99)	0.001*
rs11650072	C/T	61 (31)	27 (52)	2.38 (1.29-4.38)	201 (36)	42 (27)	0.65 (0.43-0.99)	0.001*
rs11079772	A/C	53 (27)	22 (42)	1.89 (1.06-3.34)	174 (31)	39 (24)	0.72 (0.47-1.11)	0.01*

[*]FDR-corrected P<0.05. [†]Major/Minor allele